

CLAIMS

1. Reagent for the identification and counting of biological cells in a sample, in particular in a blood sample, characterised in that it comprises:
 - a cell lysing agent selected from at least one detergent in a concentration sufficient to lyse specifically a given type of cell in the sample, and
 - a stain designed to mark the intracellular nucleic acids of the unlysed remaining cell.
2. Reagent according to claim 1, characterised in that the cell lysing agent comprising at least one ionic and/or non-ionic detergent in a concentration capable of lysing erythrocytes.
3. Reagent according to claim 1, characterised in that the detergent is selected from:
 - primary amines, amine acetates and hydrochlorides, quaternary ammonium salts, and trimethylethyl ammonium bromide;
 - amides of substituted diamines, diethanolamino-propylamine or diethylaminopropylamide, amides of cyclised diethylenetriamine;
 - alkylaryl sulfonates, petroleum sulfonates, sulfonated glycerides;

- cholamides, sulfobetaines;
- alkyl glycosides, saponins;
- 5 - polyoxyethylene ethers and sorbitans, polyglycol ethers.
- 4. Reagent according to claim 1, characterised in that the stain is a fluorescent type stain.
- 10 5. Reagent according to claim 1, characterised in that the stain is capable of combining specifically with the intracellular ribonucleic acid and enhancing its fluorescence once it has combined with the latter.
- 15 6. Reagent according to claim 1, characterised in that the stain is selected from:
 - thiazole orange or 1-methyl-4-[(3-methyl-2-(3H)-benzothiazolylidene)methyl]quinolinium p-tosylate,
 - 20 - thiazole blue,
 - 25 - 4-[(3-methyl-2-(3H)-benzothiazolyl-idene)methyl]-1-[3-(trimethylammonium)propyl] quinolinium diiodide,
 - 30 - 3,3'-dimethyloxacarbocyanine iodide or 3-methyl-2-[3-(3-methyl-2(3H)-benzothiazolylidene-1-propenyl]benzoxazolium iodide,
 - thioflavine T,

- thioflavine T,
 - the stains SYTO® and TOTO® (TM Molecular Probes),
 - 5 - ethidium bromide,
 - propidium iodide,
 - acridine orange,
 - 10 - coriphosphine O,
 - auramine O,
 - 15 - the stains HOECHST 33258 and HOECHST 33342,
 - 4',6-diamino-2-phenylindole dihydrochloride (DAPI),
 - 20 - 4',6-(diimidazolin-2-yl)-2-phenylindole dihydrochloride (DIPI),
 - 7-aminoactinomycin D,
 - 25 - actinomycin D, and
 - LDS 751.
7. Reagent according to claim 1, characterised in that it
30 also comprises at least one membrane penetration agent capable of promoting the penetration of the stain into the cells to be marked.

8. Reagent according to claim 7, characterised in that the agent promoting membrane penetration is an ionophore compound of the protonophore and/or antibiotic type.
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9. Reagent according to claim 1, characterised in that it also comprises at least one membrane fixing agent present in a concentration of 0.1% to 10% (w/v).
- 10 10. Reagent according to claim 9, characterised in that the membrane fixing agent comprises at least one alcohol and/or an aldehyde selected from paraformaldehyde and glutaraldehyde.
- 15 11. Reagent according to claim 1, characterised in that it also comprises at least one compound selected from a complexing agent, an inorganic salt and a buffer system.
- 20 12. Process for the identification and counting of biological cells in a sample, in particular in a blood sample, characterised in that it comprises the following operations:
- 25 - mixing and incubating the sample with a reagent according to one of claims 1 to 11 in order to effect, in a single stage, the lysis of cells of a given type, in particular erythrocytes, the staining of the intracellular nucleic acids, and
- 30 the fixing of the nucleate cells;
- measuring the resultant solution by flow cytometry using at least two measuring parameters

selected from resistive volume, axial luminous diffraction, axial luminous transmission, orthogonal luminous transfusion, and fluorescence; and

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- classifying and counting the nucleate cells in populations by means of the measured parameters.

13. Process according to claim 12, characterised in that
10 the resistivity measurement is carried out by means of at least one current selected from a continuous current and a pulsed or alternating current.
14. Process according to claim 12, characterised in that
15 the axial luminous diffraction parameter is at least one parameter selected from small angle diffraction and large angle diffraction.
15. Process according to claim 12, characterised in that
20 the classified nucleate cells are either mature or immature, normal or abnormal cells.
16. Process according to claim 12, characterised in that
25 the classification of the nucleate cells is carried out by means of a multidimensional analysis software program, with or without the use of a software or other neuronal technique.
17. Process according to claim 12, characterised in that
30 the sample is a sample of human or animal blood.

18. Process according to claim 12, characterised in that the sample is a sample of biological fluid or a suspension of cells of human or animal origin.